[0004] Treating antibody preparations with agents that might be expected to dissociate hetero-aggregates has generally proven ineffective. For example, employing high concentrations of urea, salts, or combinations of the two does not substantially dissociate IgM-contaminant hetero-aggregates (Gagnon *et al.*, *supra*). Protein A affinity chromatography with pre-elution washes of urea, alcohol, and surfactants has been indicated to reduce hetero-aggregate levels more effectively than without washes (Shukla *et al.*, *supra*), as did pre-elution washes combining urea, salt, and EDTA with protein G affinity chromatography (Mechetner *et al.*, *supra*). Anion exchange chromatography with a pre-elution wash of urea has been indicated to reduce hetero-aggregates more effectively than in the absence of a urea wash (Gagnon *et al.*, *supra*). Cation exchange chromatography has also been indicated to reduce hetero-aggregates more effectively with a pre-elution EDTA wash than without the wash (Gagnon *et al.*, *supra*). Finally, hydroxyapatite with pre-elution washes of urea and/or salt have also reduced hetero-aggregates more effectively than without such washes (Gagnon, *supra*). Despite these observations, in general, the use of dissociating agents in pre-elution washes of antibodies bound to chromatography columns has been only moderately successful.

[0005] Electropositive organic additives have been indicated for the precipitation of acidic proteins (Farhner et al., U.S. Patent Application No. 20080193981; Ma et al., J. Chromatogr. B (2010) 878:798-806; Peram et al., Biotechnol. Progr., (2010) 26:1322-1326; Glynn, in U. Gottschalk (ed.), Process Scale Purification of Antibodies, J. T. Wiley and Sons, (2009) Hoboken, 309-324), as well as for precipitation of DNA and endotoxins (Glynn supra; Cordes et al., Biotechnol. Progr., (1990) 6:283-285; Dissing et al., Bioseparation, (1999) 7 221:9-11) and inactivation of virus (Bernhardt, U.S. Patent. 5,559,250). Multivalent metal cations have also been indicated to remove DNA and endotoxin from some protein preparations (Akcasu et al., Nature, (1960) 187:323-324; Matsuzawa et al., Nucl. Acids Res., (2003) 3(3):163-164; Christensen et al., Prot. Expr. Purif., (2004) 37:468-471; Kejnovsky et al., Nucl. Acids Res., (1997) 25:1870-1871; Ongkudon et al., Anal. Chem., (2011) 83 391:13-17).

SUMMARY OF THE INVENTION

[0006] In one aspect, the present invention refers to a method of reducing the aggregate content of a sample comprising a desired protein at a conductivity value of 15 mS/cm or higher, the method comprising adding an electropositive organic additive at a non-zero concentration of 0.1% or less weight/volume. This disclosure provides in certain examples methods for reducing the amount of product-contaminant complexes and aggregates in a sample containing a desired protein species, such as a protein preparation, by treating the sample with electropositive organic additives. In certain examples, the reduction of complexes and/or aggregates is achieved with ultralow levels of electropositive organic additives. In certain examples, the invention particularly reduces the

content of aggregates that include chromatin remnants, such as nucleosomes, and/or histones, and/or DNA. In certain examples, the sample is treated at elevated conductivity values (salt concentration). In certain examples, the sample is additionally treated with an undissolved ureide and the supernatant containing the desired protein is collected. In certain examples, the sample is alternatively or additionally treated with other organic additives such as a nonionic organic polymer, organic solvent, surfactant, or ureide intended to enhance dissociation of contaminants associated with the desired protein in complexes and aggregates. In certain examples, the treated sample is subsequently exposed to solid materials bearing chemical moieties that selectively remove electropositive organic additives and potentially other chemical entities from the desired protein.

DETAILED DESCRIPTION OF THE INVENTION

[0007] Methods and kits are provided for the purification of proteins. In certain embodiments the invention provides for the reduction of protein-contaminant complexes and aggregates from preparations of antibodies or other proteins through the contact of such desired protein with an electropositive organic additive. In certain embodiments, the invention may be practiced at a range of conductivity levels from so-called physiological conditions to conductivity values up to 3 or more times such physiological conditions; such elevated conductivity levels may suppress precipitation of the desired or target protein. In certain embodiments, the invention may be practiced with ultralow concentrations of the electropositive organic additive; such as levels 10-fold to 100-fold lower than the concentrations at which such additives are applied for the purpose of mediating precipitation. The invention provides in certain embodiments the advantages of reducing the need for method development requirements which are customized to the protein of interest, improved recovery of the protein of interest, improved reproducibility of purification methods, and reducing the need for additional process steps to remove aggregates.

[0008] Embodiments disclose herein provide methods of reducing the aggregate content of a sample comprising a desired protein, the methods comprising contacting the sample with an electropositive organic additive and an undissolved ureide.

[0009] In some such embodiments methods of reducing the aggregate content of a sample comprising a desired protein at a conductivity value of 15 mS/cm or higher, the method comprising adding an electropositive organic additive at a concentration of 0.1% or less.

[0010] In one or more of the preceding embodiments, methods of reducing the aggregate content of a sample comprising a desired protein, the sample having a conductivity value of 5 mS/cm or higher, comprising adding an electropositive organic additive at a concentration of 0.1% or less, in

CLAIMS

1. A method of reducing the aggregate content of a sample comprising a desired protein at a conductivity value of 15 mS/cm or higher, the method comprising adding an electropositive organic additive at a non-zero concentration of 0.1% or less weight/volume.

2. The method of claim 1, wherein the electropositive organic additive comprises one selected from the group consisting of ethacridine, chlorhexidine, polyethyleneimine, methylene blue, and benzalkonium chloride.

3. The method of any one of claims 1 to 2, wherein a salt is added to a level to substantially prevent precipitation of the desired protein.

4. The method of any one of claims 1 to 3, wherein the desired protein comprises one selected from the group consisting of an antibody, a clotting factor, a peptide hormone, and a recombinant protein.

5. The method of any one of claims 1 to 4, wherein more than one electropositive organic additive is present; optionally wherein the aggregate concentration of the more than one electropositive organic additive is 1% or less weight/volume or wherein the aggregate concentration of the more than one electropositive organic additive is 0.1% or less weight/volume.

6. The method of any one of claims 1 to 5, wherein allantoin is present at a concentration in range of from about 0.56% weight/volume to about 1% weight/volume.

7. The method of any one of claims 1 to 6, wherein ureide is added to the sample before addition of the electropositive organic additive.

8. The method of any one of claims 1 to 7, wherein the sample comprises a conductivity that is adjusted to a sufficiently high level to counteract substantial loss of the desired protein through precipitation.

9. The method of any one of claims 1 to 8, wherein the conductivity of the sample is adjusted before addition of the electropositive organic additive.

10. The method of any one of claims 1 to 9, wherein an organic solvent is present.

11. The method of claim 10, wherein the organic solvent comprises one selected from the group consisting of ethanol, ethylene glycol, and propylene glycol.

12. The method of claims 10 or 11, wherein the organic solvent comprises a concentration in a range of from about 1% or greater, weight/volume.

13. The method of any one of claims 1 to 12, wherein a soluble ureide is present; optionally wherein the ureide is urea.

14. The method of any one of claims 1 to 13, wherein a surfactant is present; optionally wherein the surfactant comprises a nonionic surfactant or a zwitterionic surfactant.

15. The method of claim 14, wherein the nonionic surfactant comprises one selected from the group consisting of Tween and Triton.

16. The method of claim 14, wherein the zwitterionic surfactant comprises one selected from the group consisting of CHAPS, CHAPSO, and octaglucoside.

17. The method of any one of claims 14 to 16, wherein the surfactant is present at a non-zero concentration less than 1%.

18. The method of any one of claims 1 to 17, wherein a chelating agent is present; optionally wherein the chelating agent is positively charged.

19. The method of any one of claims 1 to 18, wherein more than one of the following is present: (a) an organic solvent, (b) an organic polymer, (c) a ureide, (d) a surfactant, (e) a chelating agent, and (f) a salt.

20. The method of any one of claims 1 to 19, wherein the contacted sample is exposed to one or more solids to remove at least one electropositive organic additive before subsequent processing steps to fractionate the desired protein from remaining contaminants.